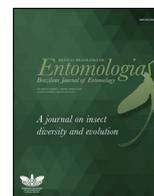




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Gene Flow Patterns of the *Aedes aegypti* (Diptera: Culicidae) Mosquito in Colombia: a Continental Comparison Suggests Multiple Invasion Routes and Gene Exchange

Bryan Steven Valencia-Marín^{1,2} , Oscar Alexander Aguirre-Obando^{1,2},
Mário Antônio Navarro-Silva^{1,*}

¹Universidade Federal de Paraná, Departamento de Zoologia, Setor de Ciências Biológicas, Laboratório de Morfologia e Fisiologia de Culicidae e Chironomidae, Curitiba, PR, Brasil.

²Universidad del Quindío, Escuela de Investigaciones en Biomatemática, Armenia, Quindío, Colombia.

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ABSTRACT

In Colombia *Aedes aegypti* is present in 80% of the country up to 2,300 m; however, little is known of its genetic relations within a country context and, hence, within a broader context, for example, America. The aforementioned, herein, analyzed the gene flow within a context of the Americas, its directionality and genetic diversity of the mitochondrial lineages in the *A. aegypti* populations for Colombia. This called for the use of the sequences for *A. aegypti* available of the mitochondrial ND4 gene in the GenBank for Colombia and the American continent. No presence was found of nuclear mitochondrial pseudogenes (NUMTs) for Colombia. It is estimated that in Colombia the gene flow of the *A. aegypti* populations is occurring from the southeast and northeast toward the center of the country. In comparison with the mitochondrial sequences for America, the vector's haplotypes in Colombia suggest connections between the populations of mosquitoes from the south with those from the north of the continent. The gene flow model at continental scale suggests bidirectional connections between the populations from the north of the continent with those from the south, while at South American scale it proposes the gene flow in all the directions with respect to the Colombian. The Colombian *A. aegypti* vector monitoring and control strategies must pay special attention to the vector's points of entry into Colombia related with Peru, Venezuela, Brazil, Mexico, and North America to avoid the entry of populations with characteristics like resistance to insecticides or vector competition.

Introduction

Aedes (Stegomyia) aegypti (Linnaeus, 1762) (Diptera: Culicidae) is the primary dengue, chikungunya, and Zika vector in the Americas (WHO, 2013, Weaver, 2014; Yakob and Walker, 2016), diseases about which there are no effective vaccines and, thereby, the most viable form to diminish its circulation is the use of chemical insecticides (Kantor, 2018). Nevertheless, excessive use of insecticides has generated globally resistance to most of the products used to control this species (WHO, 2016). *A. aegypti* originated in Africa and it is believed that its introduction to the tropical and subtropical zones of the Americas and Europe took place during the 15th and 17th centuries (Lounibos, 2002; Brown et al., 2011; Moore et al., 2013). For Colombia, it is suggested that *A. aegypti* arrived through the slave trade (OPS, 1992). In 1950, *A. aegypti* was already distributed in most of the Colombian territory; however, between 1952 and 1960 it was considered eradicated in most of the country (except for Cúcuta, Norte de Santander). By 1971, the

vector had re-infested the port cities and plains of northern Colombia (Groot, 1980). In the Americas, the *A. aegypti* eradication campaign, and therefore the yellow fever, began by the Rockefeller Foundation in 1918 (Dallimore et al., 2020) and was reformulated in 1947 by PAHO, getting for 1958 to eradicate the species in much of America and South America, including Colombia and its neighboring countries, Ecuador, Peru, Brazil and Venezuela (Magalhães, 2021). However, the late vector control actions carried out in North America (initiated in 1964 and ended in 1969) led to the reinfestation of the mosquito from there to the center and south of the continent (Magalhães, 2016).

After the vector's re-infestation, three important dengue epidemics occurred in Colombia: in 1971 (450-thousand cases due to presence of dengue 2 serotype in northern Colombia); 1976 (200-thousand cases due to presence of dengue 3 serotype in central Colombia); and in 1977 (770-thousand cases of the dengue 1 serotype throughout Colombia), where special care was assigned to the central-eastern zone of the country due to re-infestation in 1978 (Groot, 1980). Thereafter, two new arboviruses, chikungunya (Martínez et al., 2015) and Zika

*Correspondence author:

E-mail: mnavarro@ufpr.br (MA Navarro-Silva)

(Rico-Mendoza et al., 2019), emerged in the country, causing important epidemics since their appearance. In Colombia, the species is distributed up to 2,300 m (Ruiz-López et al., 2016) representing 80% of the territory (OPS, 2011) and, with this, dengue, chikungunya, and Zika manage to circulate in 95% of Colombia (Rodríguez et al., 2003; Rodríguez and de La Hoz, 2004; Badii et al., 2007; WHO, 2013; Rico-Mendoza et al., 2019). However, in spite of efforts by the vector control programs, outbreaks of these arboviruses are still common in Colombia (Martínez et al., 2015; Guagliardo et al., 2019b).

Due to the aforementioned, understanding the patterns of structure and gene flow between the vector's populations is fundamental for the rational development of vector control programs (Urdaneta-Marquez and Failloux, 2011). For example, in South America, new alternatives are being evaluated, as is the case with the release of mosquitoes infected with *Wolbachia* (populations refractory to dengue, chikungunya, and Zika) or sterile mosquitoes (populations that once they reproduce diminish the population size) (Li and Ai, 2020; Velez et al., 2020). However, in any of both techniques, it is expected that the populations released interbreed with natural populations and these, in turn, disseminate the trait introduced into the population (Aliota et al., 2016) and undoubtedly to ensure that this happens it is necessary to know the structure and gene flow patterns where they will be released (Bosio et al., 2005).

The population structure and gene flow of the *A. aegypti* populations is studied through molecular markers, microsatellites, or single nucleotide polymorphisms (SNPs), which permit analyzing the populations within a contemporary context and in a finer scale (*i.e.*, a location), although also used in bigger geographic scales (*i.e.*, between countries) (Marcombe et al., 2013; Olanratmanee et al., 2013; Monteiro et al., 2014; Rašić et al., 2014). Recently, it is also possible to access the complete nuclear or mitochondrial genomic information (Pollett et al., 2020). Notwithstanding the aforementioned, using Mitochondrial DNA (mtDNA) as molecular marker has been widely applied in genetic studies of *A. aegypti* populations from different geographic points at small and large scale and in endemic regions for dengue, chikungunya, or Zika (Gonçalves et al., 2012; Seixas et al., 2013). However, its use should be cautious because nuclear mitochondrial pseudogenes (NUMTs) can be amplified, limiting its reliability in the inferences carried out due to distinct genealogies and evolutionary histories (Black and Bernhardt, 2009; Hlaing et al., 2009).

Among the mitochondrial molecular markers used to analyze the population structure and gene flow in *A. aegypti*, there is the mitochondrial ND4 gene, which encodes subunit 4 of the NADH dehydrogenase enzyme. The mitochondrial ND4 gene has been widely used in genetic studies of populations conducted in Brazil (Twerdochlib et al., 2012), Bolivia (Paupy et al., 2012), Peru (Yáñez et al., 2013), Venezuela (Urdaneta-Marquez and Failloux, 2011), and Mexico (Gorrochotegui-Escalante et al., 2002). For Colombia, the gene structure of the dengue vector is still poorly known. In Colombia, data available on genetic diversity were obtained by using random amplification of polymorphic DNA (RAPD) (Ocampo and Wesson, 2004; Mejía et al., 2011) and mtDNA (Caldera et al., 2013; Aguirre-Obando et al., 2015), using molecular markers of the RAPD and mtDNA type. In both cases, the results of these studies demonstrate genetic structuring in the populations. In spite of this, no work exists that analyzes and compares the mitochondrial lineages available for the vector in America with the mitochondrial lineages identified for Colombia. Consequently, this work sought to analyze within a continental context the gene flow of the mitochondrial lineages presents in the natural populations of *A. aegypti* of Colombia.

Materials and methods

To estimate the structure, gene flow, and potential directionality of the gene flow of the vector populations in Colombia with respect to

the rest of the Americas, the work used sequences of the mitochondrial ND4 gene published for Colombia (Caldera et al., 2013; Aguirre-Obando et al., 2015) and America (Gonçalves et al., 2012). The publications for Colombia were obtained from a prior search in Scopus and Google Scholar by using the following criteria "*Aedes aegypti*" followed by DNA "Colombia", AND "genetic diversity", OR "gene flow", "gene structure" and "mtDNA". It considered all the databases and years. The origin (country and location) of the sequences, frequency, numbering and access numbers to GenBank are shown in Table 1.

Sequences referring to the American continent correspond to five countries: Mexico-North America, Colombia, Brazil, Venezuela, and Peru and were taken from Gonçalves et al., (2012). The sequences analyzed and compiled by Gonçalves et al. (2012) for America have as main characteristic the lack of NUMT. The sequences obtained were aligned by using the MAFT software, version 7, to detect haplotypes (H) present in them (Katoh and Standley, 2013), and these were numbered based on their frequency, thus, the most frequent was H1, followed by H2 and so on. The H found in Colombia followed the same numbering system, adding "Col" at the end (*i.e.*, Hn-Col). In addition, to reduce the error caused by the presence of NUMT in the sequences published for Colombia, two analyses were performed: **1.** A search was made for heterozygous sites in the sequences and additional stop codons and, **2.** The H were compared with the NUMT list verified by (Hlaing et al., 2009), Hlaing et al. (2009), and Black and Bernhardt (2009). If any NUMT was found, it was removed from the analysis and reported. Bearing in mind the H listed in Table 1, two haplotype networks were constructed: one for the departments of Colombia, Colombian scale, and another for the American continent (including Colombian H; continental scale). Both were constructed through the R software, version 3.5.1, using the Pegas package (Paradis et al., 2020) and the haplonet function based on the parsimony probability calculation (Templeton et al., 1992). The networks were processed graphically in Inkscape (www.inkscape.org).

Estimation of the nucleotide diversity (π), haplotypic diversity (H_d), and the D Tajima neutrality tests were estimated by location and country by using the Pegas and Strata packages in the R software.

With the H for Colombia and the Americas, the jModelTest - version 2.1.1 - was used to search for the best evolutionary model, selected through the Akaike information criterion (AIC) (Darriba et al., 2012). Thereafter, the model selected was implemented in Beast2 software (Bouckaert et al., 2014) to obtain the phylogenetic tree through Bayesian inference using the following parameters: use of a strict clock model, a Bayesian horizon coalescing model, executed with 10-million generations for the Markov Chain Monte Carlo (MCMC) with sampling every 1,000 generations and pre-burn in 10-thousand chains.

To infer the directionality of the gene flow among the *A. aegypti* populations of Colombia at continental and South American scales, two migration scenarios were tested by using a Bayesian coalescing approach in Migrate-N 4.21 (Beerli, 2004). The first scenario, at continental scale, took as comparison nodes the populations grouped for Mexico-North America, Venezuela, Peru, Brazil (Manaus, Porto Velho - Rio Branco, Belem, Boa Vista, and South-eastern Brazil), and Colombia (Sucre and Quindío). This examined six hypotheses of gene-flow models: panmixia hypothesis, total migration, two staggered stages (stepping-stone 1 and stepping-stone 2), gene flow from the north to the south or migration hypothesis from the south to the north (Fig. 1a). The second scenario, at South American scale, took as comparison nodes populations grouped for Peru, Venezuela, and south-eastern Brazil. For populations located in the Brazilian Amazon (Manaus, Porto Velho - Rio Branco, Belem, and Boa Vista) and Colombia (departament of Sucre and Quindío) these were used as independent nodes (Fig. 1b). This scenario tested five hypotheses of gene-flow models: total-migration hypothesis, two staggered scenarios (stepping-stone 1 and stepping-stone 2), gene flow from the north of South

Table 1
Haplotypes and their frequency available for *Aedes aegypti* from America, including Colombia.

| Hap | Continent | | | Brazilian | | | | | | Colombia | | GenBank | Citation |
|-------|-----------|------|------|-----------|------|----|-------|------|----|----------|---------|------------|--------------------------------------|
| | VZ | Peru | M-NA | MAO | BrAM | BV | PV-RB | SEBr | BL | Sucre | Quindio | | |
| H1 | 94 | 36 | 330 | 1 | 19 | 0 | 16 | 11 | 1 | 71 | 10 | EU650409.1 | Lima Júnior and Scarpassa (2009) |
| H2 | 99 | 0 | 9 | 22 | 30 | 7 | 0 | 2 | 1 | 38 | 0 | EU650415.1 | Lima Júnior and Scarpassa (2009) |
| H3 | 6 | 0 | 8 | 0 | 3 | 0 | 1 | 3 | 2 | 0 | 0 | AY906852.1 | Paduan and Ribolla (2008) |
| H4 | 10 | 0 | 11 | 1 | 6 | 0 | 4 | 0 | 1 | 0 | 0 | EU650407.1 | Lima Júnior and Scarpassa (2009) |
| H5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | JQ303018 | Gonçalves et al. (2012) |
| H6 | 85 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | EU650413.1 | Lima Júnior and Scarpassa (2009) |
| H7 | 331 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | DQ176835.2 | Bracco et al. (2007) |
| H8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | EU446272.1 | Burugu et al. (2008) |
| H9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | AY906840.1 | Paduan and Ribolla (2008) |
| H10 | 0 | 15 | 355 | 0 | 9 | 2 | 1 | 47 | 6 | 0 | 0 | EU650414.1 | Lima Júnior and Scarpassa (2009) |
| H11 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 1 | 0 | 0 | EU650412.1 | Lima Júnior and Scarpassa (2009) |
| H12 | 0 | 0 | 55 | 1 | 1 | 0 | 0 | 3 | 0 | 1 | 0 | DQ176840.2 | Bracco et al. (2007) |
| H13 | 0 | 0 | 26 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | DQ176831.2 | Bracco et al. (2007) |
| H14 | 0 | 0 | 283 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334858.1 | Gorochotegui-Escalante et al. (2002) |
| H15 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334850.1 | Gorochotegui-Escalante et al. (2002) |
| H16 | 0 | 0 | 214 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334851.1 | Gorochotegui-Escalante et al. (2002) |
| H17 | 0 | 0 | 93 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334852.1 | Gorochotegui-Escalante et al. (2002) |
| H18 | 0 | 0 | 139 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334854.1 | Gorochotegui-Escalante et al. (2002) |
| H19 | 0 | 0 | 130 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334864.1 | Gorochotegui-Escalante et al. (2002) |
| H20 | 0 | 0 | 154 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334859.1 | Gorochotegui-Escalante et al. (2002) |
| H21 | 0 | 0 | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334865.1 | Gorochotegui-Escalante et al. (2002) |
| H22 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334848.1 | Gorochotegui-Escalante et al. (2002) |
| H23 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334849.1 | Gorochotegui-Escalante et al. (2002) |
| H24 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334857.1 | Gorochotegui-Escalante et al. (2002) |
| H25 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | EU650416.1 | Gorochotegui-Escalante et al. (2002) |
| H26 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334861.1 | Gorochotegui-Escalante et al. (2002) |
| H27 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334847.1 | Gorochotegui-Escalante et al. (2002) |
| H28 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | AF334862.1 | Gorochotegui-Escalante et al. (2002) |
| H29 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | EU650417.1 | Lima Júnior and Scarpassa (2009) |
| H30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 20 | KF241756 | Aguirre-Obando et al. (2015) |
| H31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | JN896656 | Caldera et al. (2013) |
| H32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 11 | KF241755 | Aguirre-Obando et al. (2015) |
| H33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | JN896678 | Caldera et al. (2013) |
| H34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | JN896686 | Caldera et al. (2013) |
| H35 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | JQ303017 | Gonçalves et al. (2012) |
| Total | 626 | 51 | 1983 | 27 | 74 | 11 | 22 | 77 | 13 | 145 | 41 | | |

BrAM (Brazilian Amazon), SEBr: Southeastern Brazil; PE: Peru; VZ: Venezuela; M-NA: Mexico - North America; MAO: Manaus; BL: Belém; BV: Boa Vista; PV - RB: Rio Branco - Porto Velho.

America to the south and gene flow from the south of South America to the north (Fig. 1b). In both scenarios, the Bayesian search strategy was conducted by using the following parameters: 2×10^5 generations, number of replicates = 4, burn = 1000, using a static heating scheme with four temperatures (temperatures: 1.0, 1.5, 3.0, and 100,000). The population sizes were assumed similar (that is, θ estimated). For DNA sequences, Migrate-N assumes an F84 model of nucleotide substitution (Kishino and Hasegawa, 1989; Felsenstein and Churchill, 1996), with or without variable substitution rates with gamma distribution between sites. The best test model was selected in each scenario evaluated according with the Log Bayes factor (LBF) based on the marginal accuracy of the Bezier approximation score generated for the models from each scenario.

Results

In all, for the *A. aegypti* populations from the Americas in the GenBank, the work obtained 2,996 sequences from the ND4 gene that after aligning and cutting had a length of 395 pb. These are distributed in the following manner: Mexico - North America (64.58%), Venezuela

(20.37%), Peru (1.66%), Brazil (7.32%; South - Eastern Brazil (2.50%), Brazilian Amazon (2.40%), Manaus (0.87%), Rio Branco- Porto Velho (0.72%), Belém (0.45%), Boa Vista (0.35%)), and Colombia (6.05%). For Colombia, sequences were obtained from the departments of Sucre (4.72% distributed in the municipalities of: Sincelejo (2.03%), Guaranda (1.33%), Corazal (0.73%) and Sampues (0.70%)) and Quindio ((1.33% distributed in the municipalities of: Armenia (0.40%), Montenegro (0.33%), Quimbaya (0.33%) and Barcelona (0.33%)).

Table 1 displays the distribution and H number of the ND4 gene for *A. aegypti* in the Americas, including Colombia. The study observed 35 H without presence of NUMTs, of which eight are in the Colombian populations. In general, H1 is the most frequent (19.20%) and is distributed in all the countries. The haplotype network at continental level suggests that the Colombian populations from the departments of Sucre and Quindio are connected with populations of mosquitoes from Peru, Venezuela, southeastern Brazil, the Brazilian Amazon, and Mexico-North America (Fig. 2a). In turn, the haplotype network at Colombian level suggests that H1-Col is the most frequent and ancestral H, located in the departments of Quindio and Sucre (Fig. 2b).

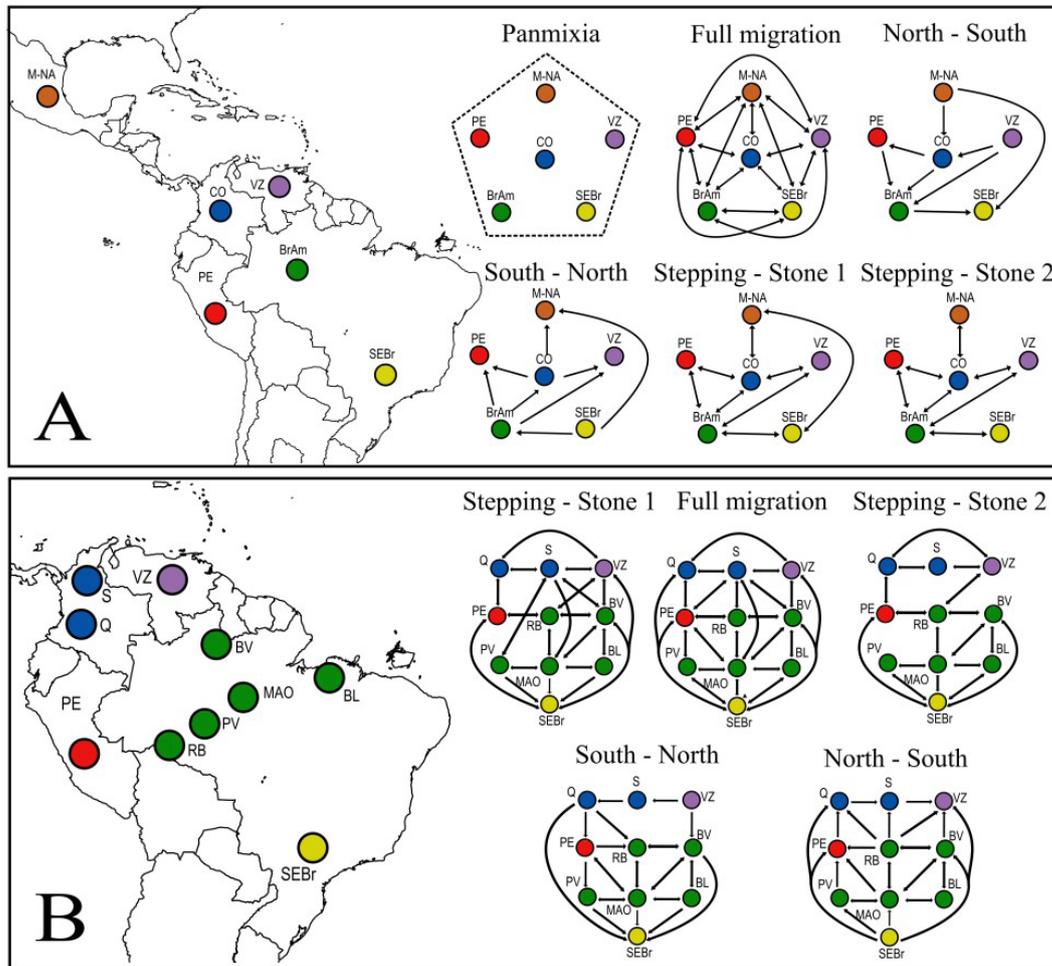


Figure 1 Geographic location of the *A. aegypti* populations included in this study and gene-flow models evaluated. In both graphics, circles indicate the populations and arrows represent the gene flow between populations. **A**) Scale at American continent level ($N = 2,996$ specimens from six locations: Mexico – North America (M-NA), Venezuela (VZ), Peru (PE), Brazilian Amazon (BrAm), southeastern Brazil (SEBr), and Colombia (CO)). **B**) Scale at South America level ($N = 1,083$ specimens from six locations: Venezuela, Peru, Brazilian Amazon (5 locations; Brazilian Amazon (BrAm), Manaus (MAO), Belém (BL), Boa Vista (BV), Rio Branco (RB), Porto Velho (PV)), Southeastern Brazil (SEBr) and Colombia (2 locations; Sucre (S), Quindio (Q))).

Table 2 shows results of haplotypic diversity (H_d), nucleotide diversity (π), and neutrality test by countries and by locations. In general, the H_d from the Americas is 0.606 ± 0.14 (mean \pm SD), while by countries the greater diversity occurred in Mexico-North America (0.731 ± 0.001), followed by Venezuela (0.655 ± 0.000), Colombia (0.648 ± 0.141), Brazil (0.595 ± 0.010), and Peru (0.423 ± 0.002). The π at Americas level was 0.008, while by countries it varied between 0.002 (Mexico-North America) and 0.017 (Brazilian Amazon). The Tajima D neutrality test (D) at Americas level indicates that most of the *A. aegypti* populations are in constant expansion, showing negative values in most cases ($P < 0.05$).

The migration scenario at continental scale suggests the hypothesis of the *stepping-stone* 1 gene-flow model with the highest subsequent probability (Model probability; 0.999, Log; -914.836 and LBF; 189.900) (Table 3), suggesting a connection of the bidirectional gene flow between the populations from Mexico-North America with the Colombian populations and connection between the populations from South America (Peru, Venezuela, Brazilian Amazon and southeastern Brazil) with the Colombian populations. A trampoline connection is shown between Mexico-North America with mosquito populations from southeastern Brazil (and from this population to the rest of the populations from South America) (Fig. 3a).

The migration scenario at South American spatial scale suggests that the hypothesis of the *full-migration* model had the highest

subsequent probability (Model probability; 0.999, Log; -725.010, LBF; 0.073) (Table 3), indicating that gene flow among the populations from South America with respect to the Colombian populations (Sucre and Quindio) occurs in all the directionalities (Fig. 3b).

The phylogenetic analysis indicates that the 35 American H are grouped into two clades, I and II (Fig. 4). Clade I groups six of the eight H observed in the populations of Colombia (H1, H2, H12, H30, H31, H32, H33, and H34), seven H exclusive for Mexico-North America (H15, H16, H17, H19, H22, H23, H26) and four H widely disseminated in the Americas (H12, H4, H3, and H1). In turn, clade II contains haplotypes distributed in populations from Brazil, Venezuela, Mexico-North America, Peru, and two Colombian H. The terminals of the phylogenetic tree, represented and differentiated with blue, red, and green, indicate the H endemic for Mexico-North America, Colombia, and Brazil, respectively.

Discussion

The mitochondrial haplotypes detected for Colombia come from vector populations from the departments of Quindio (1,845 km², Latitude: 4.533872, Longitude: -75.676974) and Sucre (10,670 km², Latitude: 4.525659, Longitude: 75.648716). The department of Quindio is located in the central-eastern zone of Colombia (Andean region)

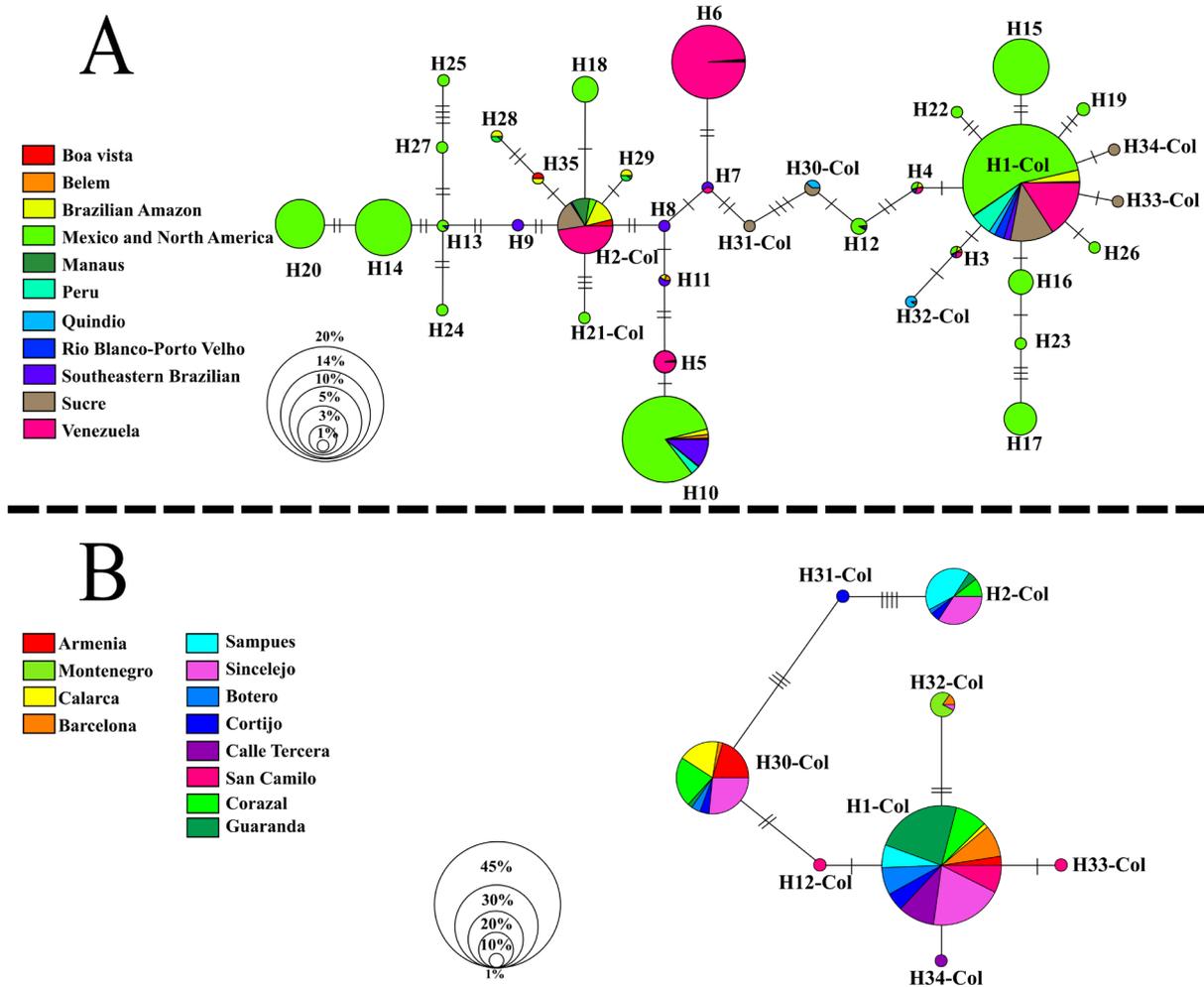


Figure 2. Haplotype network for the mitochondrial ND4 gene from natural *A. aegypti* populations from: **A.** American continent, including sequences for Colombia; **B.** Colombia. In both haplotype networks, the size of each circle is proportional with the haplotypic frequency. The number next to each circle indicates the haplotype number.

Table 2
Results of genetic diversity and neutrality tests for *Aedes aegypti* populations from America.

| Country | Localidad | Number of sequences | Genetic diversity | | | Neutrality test | |
|--------------------|-------------------------------|---------------------|----------------------|---------------|-----------|-----------------|---|
| | | | Number of haplotypes | <i>Hd</i> | π (%) | Tajimas' D | |
| Colombia | Sucre | 145 | 8 | 0,650 ± 0,000 | 0,007 | -2,604 | * |
| | Quindio | 41 | 3 | 0,646 ± 0,002 | 0,006 | -2,208 | * |
| Brazil | Brazilian Amazon ^a | 74 | 12 | 0,755 ± 0,001 | 0,017 | 0,361 | |
| | Manaus | 27 | 6 | 0,341 ± 0,013 | 0,007 | -3,151 | * |
| | Rio blanco-Porto Velho | 22 | 4 | 0,454 ± 0,013 | 0,003 | -2,512 | * |
| | Belem | 14 | 7 | 0,813 ± 0,008 | 0,016 | -2,147 | * |
| | Boa Vista | 11 | 4 | 0,600 ± 0,023 | 0,005 | -4,396 | * |
| | Southeast of Brazil | 77 | 11 | 0,606 ± 0,003 | 0,003 | -2,504 | * |
| Mex - Nort America | | 1984 | 22 | 0,731 ± 0,001 | 0,002 | -0,475 | |
| Peru | | 51 | 2 | 0,423 ± 0,002 | 0,013 | 2,669 | * |
| Venezuela | | 626 | 7 | 0,655 ± 0,000 | 0,012 | -1,577 | |

^a Manaus, Rio Branco - Porto Velho, Belem, Boa Vista are part of the Brazilian Amazon; * significant difference (p < 0.05).

and is divided into 12 municipalities, with Armenia (1,573 m) as its capital city. In turn, the department of Sucre is located to the north of Colombia (Caribbean region) and comprises 26 municipalities, with Sincelejo (213 m) as the capital city. Both departments, Quindio and Sucre, are separated by 520 km and are interconnected through the Pan-American highway (a roadway system that joins almost all the countries of the western hemisphere in the American continent, with

the exception of the Darién Gap in Colombia (Holmes, 1963) and the Colombian roadway system (INVIAS, 2014).

Two mitochondrial lineages were found in the populations evaluated, a pattern evidenced in prior studies (Bosio et al., 2005; Gonçalves et al., 2012; Aguirre-Obando et al., 2015; Monsalve et al., 2021). The haplotypes present in populations from Colombia and Mexico-North America and the bidirectional gene flow between these populations, suggested in

Table 3
Logarithmic marginal likelihood values, logarithmic Bayes factor, and probability models of gene-flow models on continental and South American scales.

| Stage | Model | Log(mL) | LBF | Model probability |
|---------------|----------------|-----------|-----------|-------------------|
| America | Full migration | -914,8366 | 189,90038 | 0,0000 |
| | Nort-Sur | -972,5316 | 247,5954 | 0,0000 |
| | Sur-Nort | -921,2833 | 196,3471 | 0,0000 |
| | Panmitico | -948,5178 | 223,5816 | 0,0000 |
| | St1 | -887,6193 | 162,6831 | 1,0000 |
| | St2 | -966,4143 | 241,4781 | 0,0000 |
| South América | Nort-Sur | -829,6100 | 104,6738 | 0,0000 |
| | Sur-Nort | -819,7800 | 94,8438 | 0,0000 |
| | Full migration | -725,0100 | 0,0738 | 1,0000 |
| | St1 | -799,5600 | 74,6238 | 0,0000 |
| | St2 | -826,6500 | 101,7138 | 0,0000 |

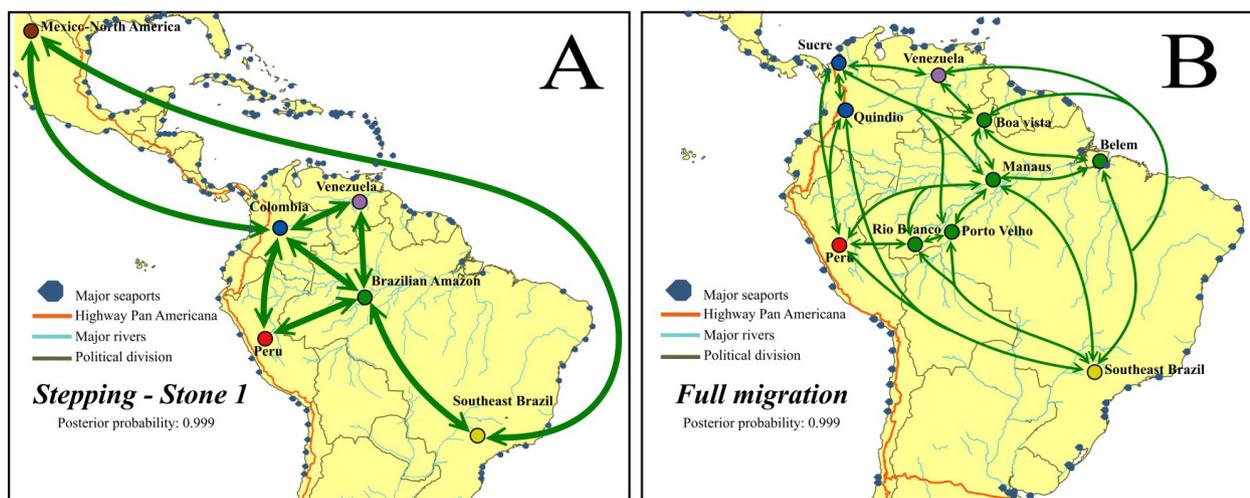


Figure 3. Representation of the gene-flow model with greater subsequent probability in the scenarios of the Americas and South America. **A.** Scale at American continent level ($N = 2,996$ specimens from six locations: Mexico–North America, Venezuela, Peru, Brazilian Amazon, southeastern Brazil, and Colombia). Within the continent level, the *Stepping Stone 1* model shows through the green vectors the way the gene flow occurs among the different populations evaluated. **B.** Scale at South America level; the *Full-migration* model indicates migration among all the populations, hence, plotting of the potential migration routes through passive land transport (Pan-American highway) and riverine paths (principal rivers).

our *stepping-stone – 1* model, could be supported by the presence of maritime routes between both countries, given that these have been associated with the global propagation of *A. aegypti*, bearing in mind population genetics studies conducted in maritime ports in Asia and Brazil (Paduan and Ribolla, 2008; Hlaing et al., 2010).

Among the mechanisms that could explain gene flow between the mosquito populations from Colombia toward Mexico–North America, there is passive migration due to human activity (Gorochotegui-Escalante et al., 2002; Aguirre-Obando et al., 2015), specifically, the essential role of maritime ports in disseminating mosquitoes of the genus *Aedes* (Futami et al., 2015; Kollars, 2017). For example, in Colombia, the Port of Buenaventura on the Pacific is the main cargo port in the country, responsible for 50% of imports in Colombia (Pérez-Valbuena, 2007); it is located 165 km from the Department of Quindío. The Port of Barranquilla on the Atlantic, the second-most important in Colombia, has a direct maritime flow with the Gulf of Mexico and is located 730 km from the department of Quindío and 415 km from the department of Sucre (Otero, 2012). There is qualitative and quantitative evidence that recognizes the fundamental role of passive transportation of maritime flow and maritime ports in the dispersion and dissemination of immature mosquito forms (Chadee, 1984; Sprenger, 1987; Halstead, 2000; Fonzi et al., 2015). For example, immature forms transported by used tires (Medlock et al., 2012) and “Wet Footed” plants, such as

Lucky Bamboo (Demeulemeester et al., 2014) have been observed. Historically, in the Americas, the transportation of tires used by sea, and subsequently by land, has favored the dispersion of *Aedes* mosquitoes (Sprenger, 1987), where it has been suggested that this was one of the main sources of entrance of mosquitoes in the reinfestation of *A. aegypti* from the United States to South America (Halstead, 2000). In Colombia, for 2021, for example, a total 4,653 tons of used tires from Mexico and USA (1,429 t), Brazil (2,397 t), Venezuela (25 t), Peru (141 t) and China (212 t) (ITC, 2022). The high plasticity of *A. aegypti* to the use of containers to deposit eggs and their ability to enter the temporary diapaus process allow them to survive to the evaporation process (draining) and long trips in small water bodies formed in small temporary ponds in the containers (Dallimore et al., 2020). Hence, it is suggested that the entry of individuals of this vector into the country from Mexico–North America to Colombia takes place through passive maritime transport. Moreover, additional genetic and population studies are suggested of the vector aimed at the Colombian ports to verify our hypothesis.

The connection of the Colombian populations with those from South America (Peru, Venezuela, and Brazil (= Brazilian Amazon + Southeastern Brazil)) may be explained by two factors: riverine connectivity and land transport. Riverine connectivity has evidenced that it plays an important role in passive transport of *A. aegypti* populations between

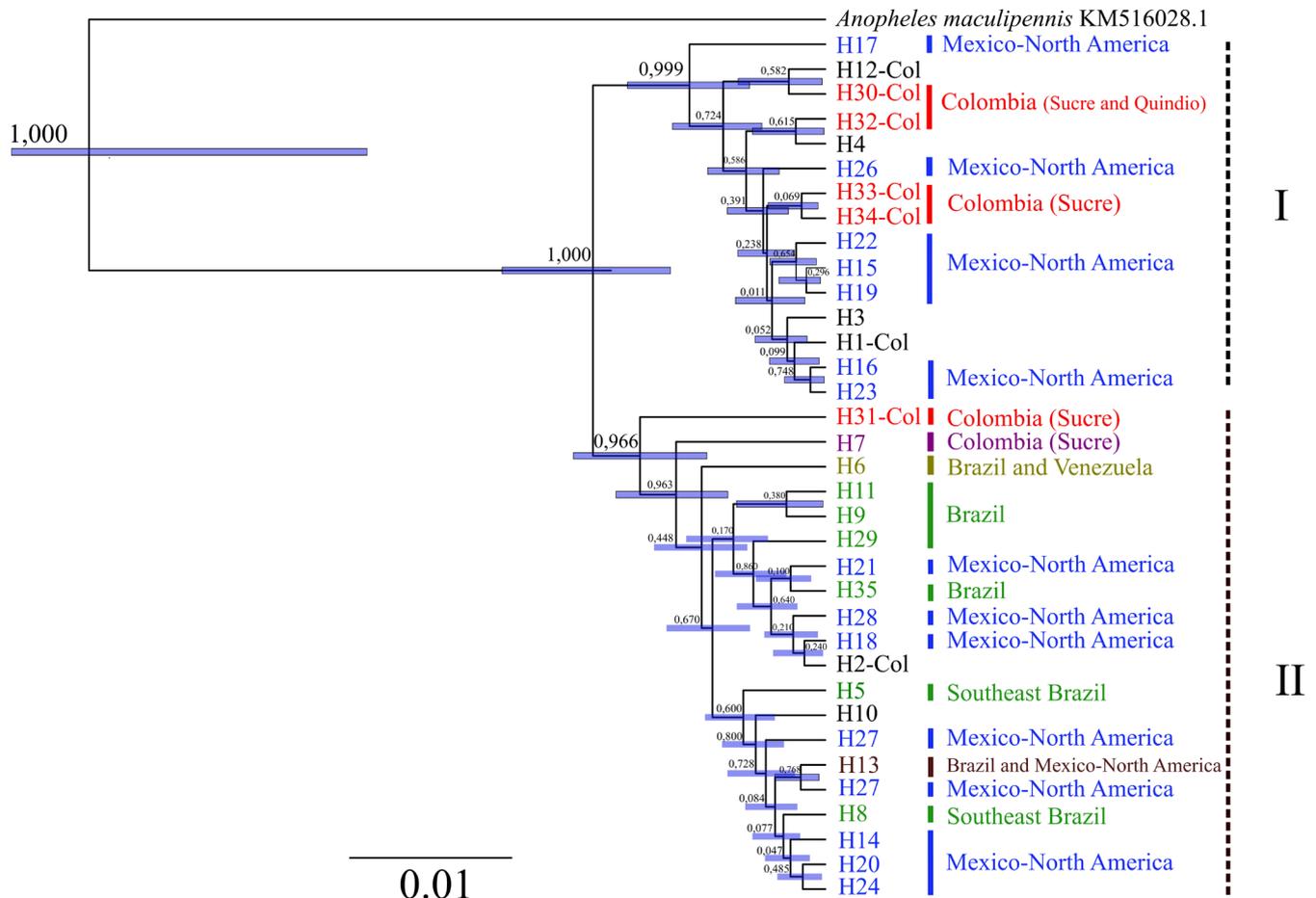


Figure 4. Phylogeny constructed through Bayesian inference estimated from the 35H found of the ND4 gene for the *A. aegypti* populations in the American continent. The blue horizontal bars above the branches reflect the 95% CI for the branch supports. The color bars (blue, green, and red) on the tree terminals indicate which haplotypes are exclusive for a specific population. The dotted lines on the right side of the tree and numbers I or II indicate to what clade each of the terminals belong. H1-Col (Colombia (Sucre and Quindio), Venezuela, Peru, M-NA, Brasil (MA-O, RBPV, SEBr, BE-L)), H4 (Venezuela, M-NA, Brasil (MA-O, RBPV, BEL)), H3 (Venezuela, M-NA, Brasil (RBPV, SEBr, BE-L)), H2-Col (Colombia (Sucre), Venezuela, Peru, M-NA, Brasil (RBPV, SEBr, BE-L)), H13 (M-NA, Brazil (SEBr)), H8 (Venezuela, Brazil (SEBr)).

Peru and the Brazilian Amazon and is supported by observation of pupae on ships and barges between these locations (Guagliardo et al., 2014, 2015, 2019a). For land transport, along the American continent is the Pan-American highway that connects and facilitates land transport among most countries in the Americas, except for a 130-km stretch in Panama, Central America. In this sense, the Pan-American highway could facilitate passive migration between populations of vector mosquitoes from Colombia to the other countries in South America, given that it is recognized that roadway connectivity (Hlaing et al., 2010; Gonçalves et al., 2012; Maffey et al., 2020), tire cargo transport (Moore and Mitchell, 1997; Bennett et al., 2019), and flow of private vehicles (Eritja et al., 2017) influence directly on the passive migration of mosquitoes from the genus *Aedes* and its associated diseases (Mahabir et al., 2012). In any scenario, whether terrestrial or riverine, both are associated globally with passive transport of immature or adult forms of the dengue vector (Morrison et al., 2006; Gonçalves et al., 2012). A mathematical model proposed by our research group, incorporating land cargo transport for *A. aegypti* and the Mayaro virus, suggests that the presence of roadways with greater flow of cargo influence on the dissemination of the vector at broad geographic scales and, consequently, contribute with the epidemiological spread of arbovirus (Valencia-Marín et al., 2020).

Existing genetic connections among the vector's populations in southeastern Brazil and the Brazilian Amazon would be based on two

facts: the first, on the history of the vector's re-infestation in Brazil after the 1955 eradication campaign, which is presumed to have started in southeastern Brazil, reaching the central-western region and regions in the northeast by mid-1980 and, finally, reaching all the other Brazilian states in 1998 (Figueiredo, 2003); the second is based on the existence of the highways BR364 (communicating São Paulo-Acre) and BR153 (Rio Grande do Sul-Belém) in Brazil, which connect the north and south regions in Brazil (Gonçalves et al., 2012). Existing connections among *A. aegypti* populations between Venezuela and the northern Brazilian Amazon (Boa Vista) are based on the presence of dengue serotypes in Boa Vista, previously only registered in Venezuela (Figueiredo, 2003; Lourenço-de-Oliveira et al., 2004; Codeço et al., 2009).

In a national scale, the gene flow of *A. aegypti* populations in Colombia, according to that found in the migration hypothesis at South American scale occurs in panmictic manner (Fig. 3b). This hypothesis is supported by recent studies with microsatellites for three Colombian populations that evidence panmictic behavior (Monsalve et al., 2021). Consequently, the vector's dispersion toward the center of Colombia could occur through land or river communication routes. Land communication would be taking place through the principal roadways in Colombia; among them, the Pan-American highway, which communicates the central-eastern region with the north of the country. It is estimated that annually close to 9.5-million t (Pérez-Valbuena, 2007) are transported from Valle del

Cauca (mainly from Buenaventura) to the four principal capital cities in Colombia (Bogotá, Cali, Medellín, and Barranquilla). Most of this cargo goes through highways in the department of Quindío, which permit roadway communication with Bogotá, Medellín, and Barranquilla.

In turn, riverine transport, principally through the Magdalena and Cauca rivers, contribute with the passive dispersion of the vector from the central-eastern region to the north of Colombia and vice versa. The Magdalena River originates on the border between the departments of Cauca and Huila and the Cauca River in the department of Cauca; both departments located in the central-western region of Colombia. The Magdalena and Cauca rivers form the first and second riverine artery in Colombia, respectively. The Magdalena River, whose principal affluent is the Cauca River, empties into the Caribbean Sea at 7.5 km from Barranquilla. The Magdalena River occupies 24% of the Colombian continental territory, where 18 departments are located and where 80% of the population inhabits and 85% of the Colombian gross domestic product (GDP) is produced (Ministerio de Ambiente, Vivienda y Desarrollo Territorial, 2010).

However, more studies are needed in different parts of Colombia to corroborate the hypotheses exposed in this document.

Conclusions

Strategies for *A. aegypti* monitoring and vector control must pay close attention to the vector's points of entry to Colombia related with Peru, Venezuela, Brazil, Mexico, and North America, to avoid the entry (and exit) of new populations with characteristics, like resistance to insecticides or vector competition to arboviruses circulating in Colombia or to new emerging arboviruses. Our results show the importance and implication of the different mechanisms and migration routes of the vector in two spatial scales, continental and subcontinental, which can be considered to implement and plan vector monitoring and control programs.

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Conflicts of Interest

The authors declare having no conflict of interests.

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